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The Relationship Between Mutagenicity and Chemical Composition of Polycyclic Aromatic Compounds From Coal Pyrolysis

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The polycyclic aromatic compounds (PAC) produced from the pyrolysis of a bituminous coal at temperatures of 1125 to 1425°K prove to be mutagenic to *S. typhimurium*, both in the presence and in the absence of postmitochondrial supernatant (PMS) prepared from Aroclor 1254-induced rat liver. Mutagenicity of the PAC samples measured in the absence of PMS exhibits little dependence on pyrolysis temperature; that measured in its presence is higher at the higher pyrolysis temperatures. However, because of the decrease in PAC yield as the temperature is raised, mutagenicity per mass of coal consumed falls with an increase in temperature if measured without PMS (−PMS) and peaks at an intermediate temperature of 1378° K if measured with PMS (+PMS). Using a new chromatographic technique, we have split each coal-derived PAC sample into two fractions: LC1, containing PAC with alkyl and O-containing substitutions and LC2, consisting of unsubstituted PAC. Substituted (LC1) fractions show no significant +PMS mutagenicity, indicating that, as a whole, the alkylated PAC in our coal pyrolysis products are not mutagenic. Only at the higher temperatures do the substituted fractions exhibit significant −PMS mutagenicity, attributed to PAC with carbonyl or etheric functionalities. The extremely low yields of the substituted PAC under the conditions where they show some activity, however, ensure that they contribute little to overall mutagenicity. In contrast to the substituted fractions, the unsubstituted (LC2) fractions display significant mutagenicity under all conditions and appear to be responsible for virtually all of the mutagenicity in these coal-derived PAC samples. In this fraction, −PMS activity is attributed to nitrogen-containing heterocyclic aromatics.

Introduction

All coal conversion processes—combustion, gasification, and liquefaction—include as their initial step pyrolysis or thermal degradation of the coal. When pyrolyzed, coal releases its constituent aromatic clusters as polycyclic aromatic compounds (PAC), some of which contain N, S, or O heteroatoms within the aromatic rings (*1-5*) or functional groups as substitutes for ring hydrogen (*3-6*). If further exposed to pyrolytic conditions, the PAC can undergo chemical transformations that lead to changes in their amount and composition.

Since many PAC are mutagenic (*7-10*) or carcinogenic (*11-13*), there have been several attempts to correlate biological activity with compound structure. Results to date indicate that biological activity is a complex function of the size and configuration of the parent PAC structure (*14-16*), the presence or absence of ring heteroatoms (*12,16*), the presence or absence of substituent groups (*8,12,17-19*), and the nature and position of such substituents (*11,13,17,20-25*). Because each of these factors influences the electron distribution within the molecules, it is logical that they should also be governing PAC behavior under pyrolytic conditions.

It is clear that molecular structure is the key factor in determining the chemical and biological activity of PAC. Since PAC from coal pyrolysis contain so many individual species with many of them isomeric, we have chosen to describe compositional changes of the PAC in terms of three structural characteristics: the degree of functional group substitution, the number of fused aromatic rings, and the presence or absence of heterocyclic nitrogen. These structural characteristics each represent major classes of mutagens.

Addressing each of these characteristics, our previous papers (*26-28*) detail the compositional changes in our coal-derived PAC that accompany variations in pyrolysis temperatures and residence time. We now

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attempt to relate pyrolysis-induced changes in the mutagenicity of the PAC to those in their chemical composition. We choose a high-volatile bituminous coal for our study as it has been shown to produce PAC of high yield (*29*) and mutagenicity (*30*).

Salmonella typhimurium forward mutation assays are run on PAC mixtures that result from pyrolysis of the coal at a constant residence time but over a range of temperatures. In addition to examining each mixture as a whole, we took advantage of a recently developed high-pressure liquid chromatographic (HPLC) technique (*31*) that enabled us to physically separate each PAC mixture into two fractions: LC1, PAC containing alkyl, phenyl, hydroxyl, carbonyl, carboxyl, cyano, or nitro functionalities; and LC2, unsubstituted PAC and PAC with ring nitrogen (PACN). Mutagenicity is measured both in the presence and absence of Aroclor 1254-induced rat liver postmitochondrial supernatant.

Experimental Equipment and Procedures

To produce the PAC of this study, 44- to 53-μm particles of PSOC 997, a Pittsburgh Seam high-volatile bituminous coal, are fluidized in argon and fed at a rate of 42 mg/min into a laminar flow, drop-tube pyrolysis furnace described elsewhere (*26,29*). An optical pyrometer is used to measure furnace temperature, which is set to values of 1100°K to 1500°K by adjustment of the electrical power input. Average gas residence time is set at 0.75 sec for these experiments.

As pyrolysis products exit the reaction zone at 5.3 std L/min, they encounter 17.1 std L/min of argon quench gas at the top of the collection probe and another 4.8 std L/min along the length of the collection probe. This latter quench gas, radially transpired through the porous walls of the inner tube, prevents deposition of the products onto the walls. The heavier species (≥ 2 rings) condense onto the surface of the soot and char during the cooling. Exiting the probe, the pyrolysis products enter a modified Andersen cascade impactor for size-separation of the solid products. Char particles, the larger of these, deposit on the first stages; aerosols (i.e., PAC-coated soot) end up on the lowest impactor stages and the Millipore Teflon filter (hole size, 0.2 μm) following the impactor. The gas temperature just downstream of the Teflon filter, as measured by a thermocouple, is 26 ± 2°C for all experiments, regardless of furnace temperature. Analysis by GC/MS/FID of gases passing through the filter reveals significant levels of hydrocarbons ranging from methane to benzene and toluene. Though specifically searched for, naphthalene and heavier aromatics are not detected in the gas phase but are found in the condensed phase. It is the condensed material that is the focus of this paper.

After all products are weighed, the char and soot are each placed in Teflon-capped, 30-mL amber glass bottles of Caledon distilled-in-glass HPLC grade dichloromethane (DCM) and sonicated for 5 min. [Work with standards (*31*) shows DCM to be a good solvent for a variety of aromatic species: polystyrenes up to molecular weight 3×10^6 and substituted PAC up to molecular weight 10^3.] The particle/liquid suspensions are passed, by syringe, through Millipore Teflon filters (hole size 0.2 μm) to remove the char or soot particles from the PAC/DCM solutions. The mass of each residue solid is taken and subtracted from that before sonication to give the mass of the PAC. Triplicate 100-μL aliquots of the PAC/DCM solutions are removed, evaporated, and weighed according to the procedure of Lafleur et al. (*32*) to verify the PAC yields. As previously documented (*33*), > 90% of the PAC condense onto the soot (as opposed to the char), so we restrict our current analysis to the soot-associated PAC.

The PAC/DCM solutions undergo a variety of chromatographic and spectroscopic analyses (*26-28*). Fully described elsewhere (*26,31*), the HPLC system used for fractionation consists of a Perkin-Elmer Series 4 quaternary solvent delivery system coupled to a Model LC-85B variable wavelength ultraviolet (UV) detector. 1.5 mL/min of DCM (same grade as above) flows through the steric exclusion column (50 cm long × 10 mm, i.d.), which is packed with 500 A Jordi-Gel polydivinylbenzene. Samples are injected through either a 100- or 200-μL Rheodyne injection loop, and a microswitch on the injector actuates the data system to ensure reproducible starting times. As demonstrated in another publication (*31*), substituted PAC elute in the first 23.9 mL; unsubstituted and nitrogen-containing PAC (PACN), afterward. For each coal-derived PAC sample, then, we collect the first 23.9 mL of column eluate as fraction LC1; the remaining eluate as LC2. The fractions are each collected in Teflon-capped, amber glass bottles and concentrated under a stream of nitrogen. Gravimetric and chromatographic analyses of the fractions from several parallel fractionations demonstrate that the system is highly reproducible.

Since DCM is toxic to bacterial cells, samples are exchanged into dimethylsulfoxide (DMSO), a less toxic solvent, before bioassay. To exchange a sample, 30 μL of DMSO are added to a DCM aliquot containing 60 μg PAC and placed under a stream of nitrogen, which vaporizes the DCM, leaving the PAC dissolved in DMSO.

The bacterial mutagenicity of the pyrolysis-derived material is determined from the *Salmonella typhimurium* forward mutation assay of Skopek et al. (*34,35*). A suspension of *S. typhimurium* strain TM677 is incubated with the test material for 2 hr at 37°C, diluted 1:5 in the phosphate-buffered saline, and plated in triplicate on selective plates in the presence of 400 μg/mL 8-azaguanine. All incubations are made in duplicate and run concurrently with positive and negative controls. Postmitochondrial supernatant (PMS) from Aroclor 1254-induced rat liver (Litton)

and appropriate cofactors are included in half of the incubations in order to provide a source of enzymes mimicking mammalian drug metabolism. Mutagenic activity detected in the presence of PMS is denoted as +PMS; that in its absence, as −PMS.

Results and Discussion

Figure 1 presents the results of the bioassays performed on the unfractionated PAC mixtures obtained from pyrolysis of the coal at five different temperatures. Plotted here are the fractions of the bacterial population that become mutants, either +PMS or −PMS, as functions of the concentration of the PAC mixtures to which they are exposed (specific mutagenicity). The horizontal line at the ordinate value of 24×10^{-5} denotes the ninety-fifth percentile of the mutant fraction exhibited historically by this particular lot of *Salmonella typhimurium* in the absence of any test substance. Points lying below the horizontal line are thus to be regarded as insignificant with regard to induced mutagenicity, since these

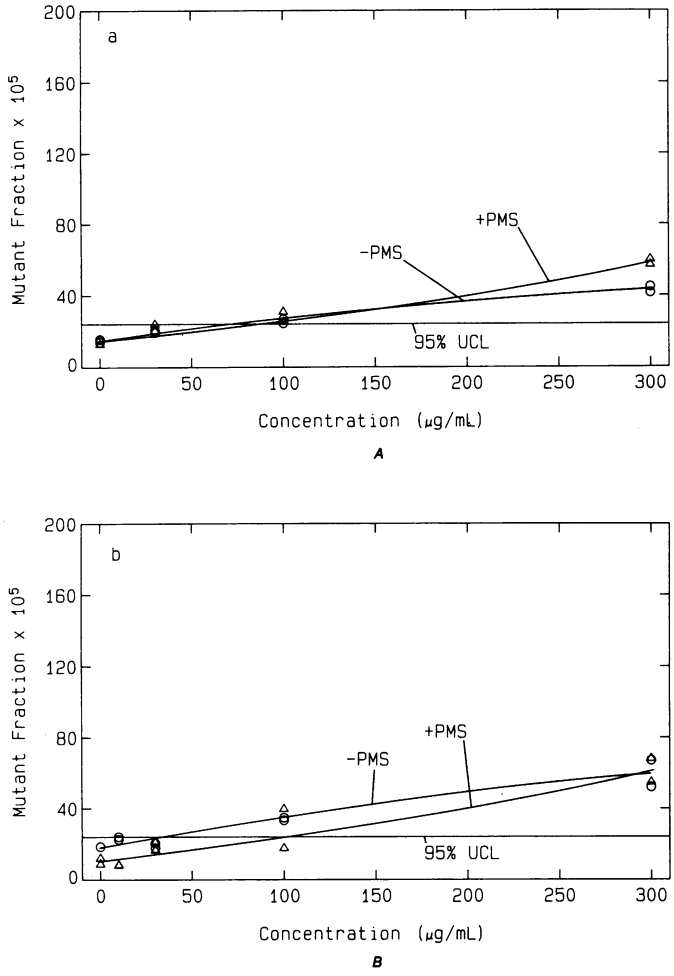

FIGURE 1. Specific mutagenicities of unfractionated PAC samples. (Δ) +PMS; (o)−PMS. Pyrolysis temperatures: (*a*) 1125°K; (*b*) 1223°K; (*c*) 1312°K; (*d*) 1378°K; (*e*) 1421°K.

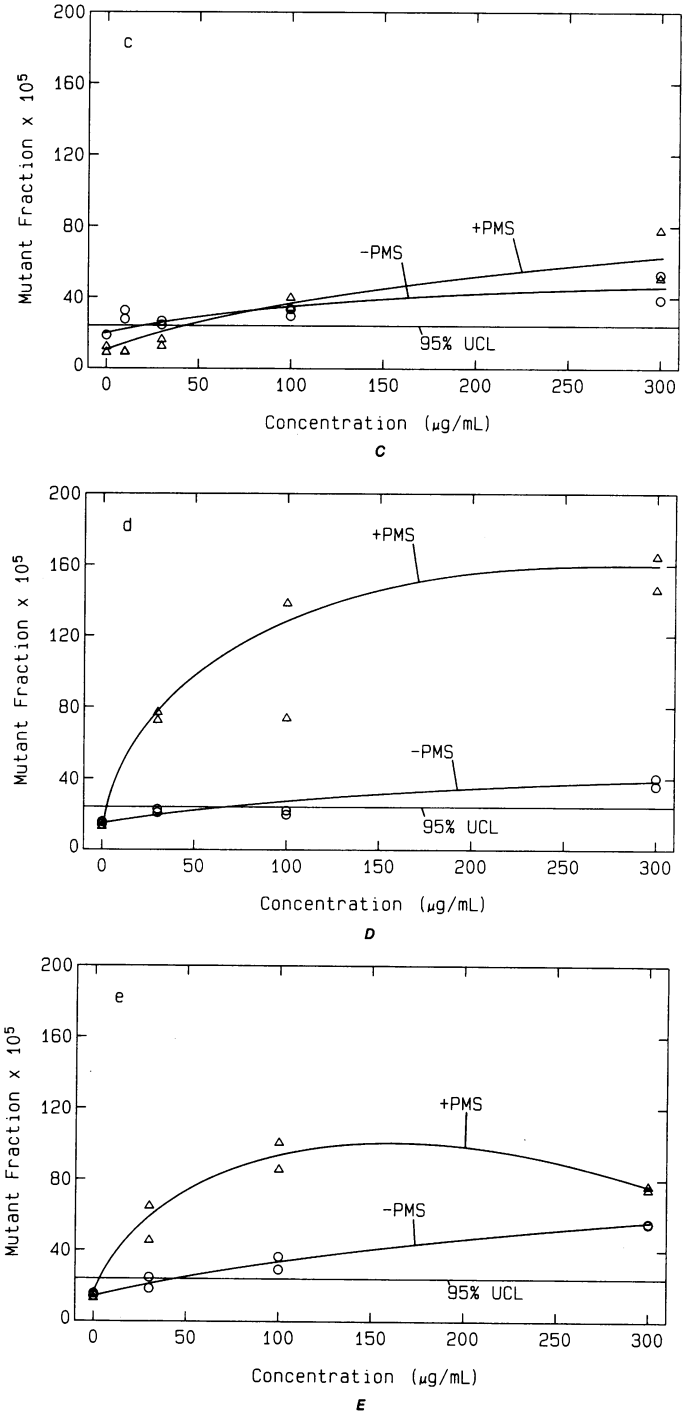

FIGURE 1. *(continued)*

levels of mutagenicity cannot be distinguished statistically from those that occur naturally for these bacteria in the absence of a test substance. Each experiment is run in duplicate, so the spread between like symbols at a given concentration gives an indication of the reproducibility of the data.

As seen in Figure 1, the PAC from all the experiments induce significant +PMS and −PMS mutagenicity within the range of concentrations investigated. (The upper value of the administered

concentration is limited by the toxicity. At high concentrations, the test substance kills a large fraction of the bacteria, leaving a small fraction from which to determine mutagenicity.) As expected, +PMS and −PMS mutagenicities increase as the concentration of the test species increases. Although differences between +PMS and −PMS mutagenicities are hard to distinguish at the lower pyrolysis temperatures, generally +PMS mutagenicity appears to be greater than −PMS mutagenicity. This distinction is particularly apparent at the higher pyrolysis temperatures, where +PMS mutagenicities become quite high (up to 160×10^{-5}. Mutagenicities measured in the absence of PMS, on the other hand, show relatively little change with pyrolysis temperature, never exceeding 70×10^{-5}.

Figure 1 shows that the +PMS specific mutagenicity of the PAC formed in coal pyrolysis increases with temperature. However, our previous work (*26,33*) also shows that the yield of PAC from coal decreases as temperature is raised. Figure 2 compares the mutagenicity of the PAC samples on the basis of the amount of coal pyrolyzed (total mutagenicity). The mutagenicities of Figure 1 are combined with the PAC yields (*31*) to obtain Figure 2, where the abscissa is concentration in terms of the amount of coal consumed. The different symbols in Figure 2 correspond to different pyrolysis temperatures. +PMS experiments appear in Figure 2*a*; −PMS, in 2*b*. Data are lacking for the low temperature experiments at high coal doses since the correspondingly large PAC doses were toxic to the bacteria. Nevertheless, Figure 2 allows some conclusions to be drawn. In the absence of PMS, for a given amount of coal consumed, it appears that the mutagenic activity from the lower temperature experiments is slightly higher than those at the higher temperatures. As temperature increases, −PMS specific mutagenicity changes little but total PAC yield decreases, so overall −PMS mutagenicity (per mass of coal) decreases. Figure 2*a*, however, shows that in the presence of PMS, the mutant fraction appears to be relatively insensitive to pyrolysis temperature, for a given amount of coal consumed, except for the 1378°K experiment. At $T <$ 1378°K, the specific +PMS mutagenicity is low, but the yield is high; at $T >$ 1378°K, the specific +PMS mutagenicity is high, but the yield is low. A peak in the overall +PMS mutagenicity thus appears at the intermediate temperature of 137°K.

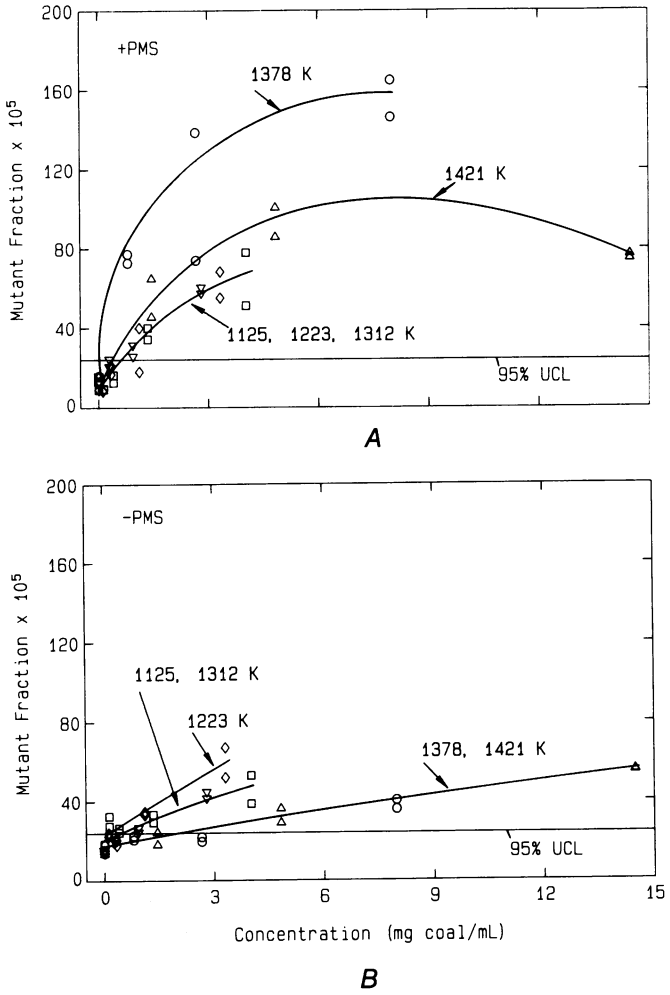

FIGURE 2. +PMS and −PMS overall mutagenicities of unfractionated PAC samples. Pyrolysis temperatures: (▽) 1125°K; (△) 1223°K; (□) 1312°K; (o)1378 °K; (△) 1421°K.

Figures 1 and 2 suggest that the +PMS and −PMS mutagens exhibit different sensitivities to pyrolysis conditions. It is important, then, to examine the differences in the types of compounds classified as +PMS and −PMS mutagens. Considering mutagenicity results of the products from a number of fossil fuels, Howard and Longwell (*36*) associate −PMS mutagenicity with polar oxygenated and nitrogen-containing PAC; +PMS mutagenicity with PAH (PAC without N, S, or O heteroatoms). The results of Alfheim et al. (*37*) suggest that nitrogen-containing PAC may also exhibit +PMS mutagenicity. Our data so far imply that as pyrolysis temperature increases, polar PAC accounts for a smaller portion; PAH accounts for a larger portion of the mutagenic activity present in the PAC. To establish the classes of compounds responsible for the mutagenicity in our samples, a new HPLC technique (*31*) was employed, allowing us to separate the PAC into two groups: LC1, PAC containing alkyl, phenyl, hydroxyl, carbonyl, carboxyl, cyano, or nitro functionalities; and LC2, which had unsubstituted PAC and PAC with ring nitrogen. Having analyzed our PAC mixtures with a variety of chromatographic and spectroscopic techniques (*26-28*), we know that LC1 for these samples is comprised mainly of alkylated PAC, with some contributions from PAC with oxygen-containing substituents; LC2 is primarily composed of unsubstituted PAH and PAC with ring nitrogen. Each HPLC fraction thus contains a +PMS and a −PMS mutagen compound class.

Prior to running bioassays on the HPLC fractions of the PAC samples, it is necessary to verify that the fractionation procedure does not introduce mutagenic activity to the samples or remove activity from them.

We have previously injected and recovered an extensive series (approximately 190) of PAC standards, many of them polar, on this HPLC column (*31,38*). There is no evidence that the column retains compounds irreversibly. Nevertheless, we have tested the integrity of the column on two of our PAC product mixtures. In the test, we compare the mutation behavior of each unfractionated sample with that of the physically combined two fractions of that sample taken from the HPLC column. The results of the test, conducted on both a low temperature and a high temperature sample, appear in Figure 3. (The bacteria used in this test are from a different lot than those used in the other experiments in this paper, so the numerical values in Figure 3 for the PAC-induced mutagenicity as well as for the 95% UCL are different from those in the other figures.) Within the precision limits of the bioassays, Figure 3 shows that there is no discernible difference between the mutagenicity of the whole samples or their corresponding combined fractions—whether +PMS or −PMS and whether low or high temperature.

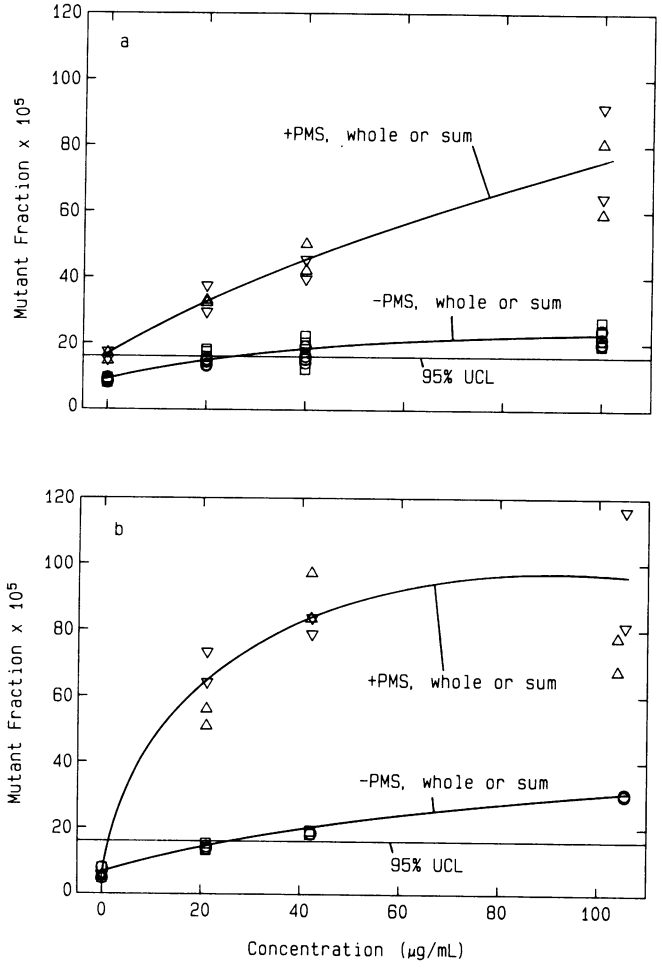

FIGURE 3. Specific mutagenicities of unfractionated PAC samples and their combined HPLC fractions. (cad148) +PMS, whole; (▽) +PMS, sum; (□) −PMS, whole; (o) −PMS, sum. Pyrolysis temperatures: (*a*) 1223°K; (*b*) 1421°K.

Having established that our fractionation procedure introducess no spurious results, we examined the results of the bioassays conducted on the two chromatographic fractions of each sample. Plotted in Figure 4 are the +PMS and −PMS mutagenicities of the LC1 (triangles) and LC2 (squares) fractions of each pyrolysis sample. Most apparent from Figure 4 is that for all pyrolysis conditions, none of the substituted (LC1) fractions exhibit appreciable +PMS mutagenicity. Since alkylated PAH would appear in this fraction and since they are known to demonstrate mutagenic behavior only in the presence of PMS, we conclude that, as a whole, the alkylated PAH produced from our coal are not significantly mutagenic, even though 2 and 9 methyl anthracenes and phenanthrenes are mutagenic in the assays employed in this study (*7*).

We also see from Figure 4 that the substituted fractions show negligible −PMS mutagenicity at the three lowest pyrolysis temperatures of 1125, 1223, and 1312°K. At 1378 and 1421°K, they show small but insignificant levels of mutagenic activity. The type of −PMS mutagens that would be present in the substituted fractions are PAC with oxygen-containing substituents. Prior Fourier transform infrared (FTIR) analyses (*26*) on these samples show that at the higher temperatures, there is evidence of both etheric C—O and carbonyl and C—O stretch but no trace of hydroxyl O—H stretch. We therefore attribute the small levels of −PMS mutagenicity in the substituted fractions to PAC with etheric and/or carbonyl functionalities. Despite the small but significant increase in mutagenic activity of the higher temperature LC1 fractions, the substituted PAC as a whole contribute inconsequentially to the overall activity of the PAC samples since the yield of the substituted PAC, as shown previously (*26*), falls drastically as pyrolysis temperature increases. At 1378 and 1421°K, less than 30% of the total PAC present is substituted, and the total PAC yield is low.

Compared to the substituted fractions, the unsubstituted (LC2) fractions for all pyrolysis temperatures exhibit substantially higher levels of mutagenicity, whether + or −PMS. The unsubstituted PAC also comprise a larger portion of the total PAC as pyrolysis temperature increases (35% of the total PAC are in LC2 at 1125°K; 75% at 1421°K.). As shown in Figure 4, +PMS mutagenicity of the unsubstituted fractions generally increases with temperature. −PMS mutagenicity of these fractions increases abruptly from 1125 to 1223°K, then gradually lessens as temperature rises further. The −PMS mutagenicity is greater than the +PMS mutagenicity for the 1125, 1223, and 1312°K unsubstituted PAC. At 1378°K, however, the two approach equality, and the +PMS mutagenicity slightly surpasses the −PMS at 1421°K.

The unsubstituted fractions are comprised of PAH, some of which are known to be +PMS mutagens; and of PACN, some of which are −PMS mutagens and perhaps +PMS mutagens as well (*4*). The ever-increasing +PMS mutagenicity of the unsubstituted

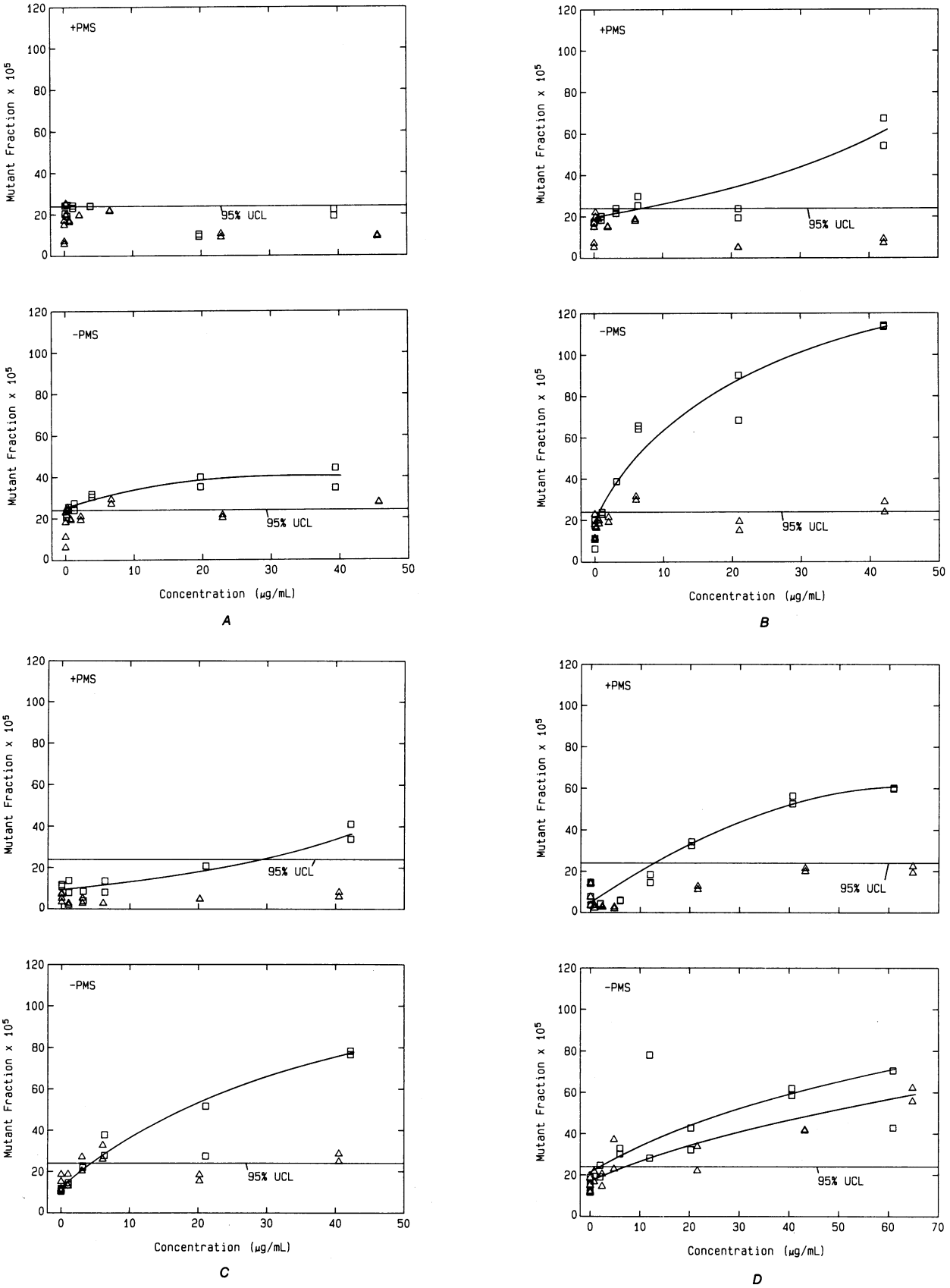

FIGURE 4. +PMS and −PMS mutagenicities of PAC fractions. (Δ) LC1; (□) LC2. Pyrolysis temperatures: (*A*) 1125°K; (*B*) 1223°K; (*C*) 1312°K; (*D*) 1378°K; (*E*) 1421°K. (*Continued on next page.*)

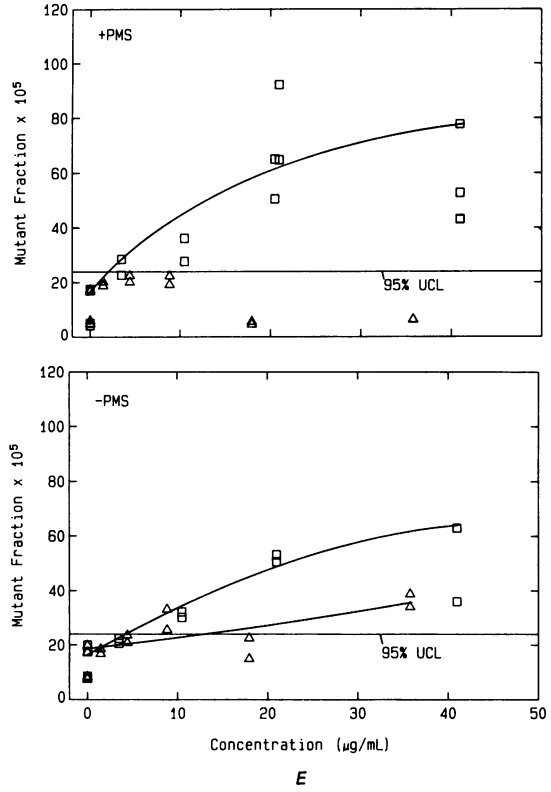

FIGURE 4. *(continued)*

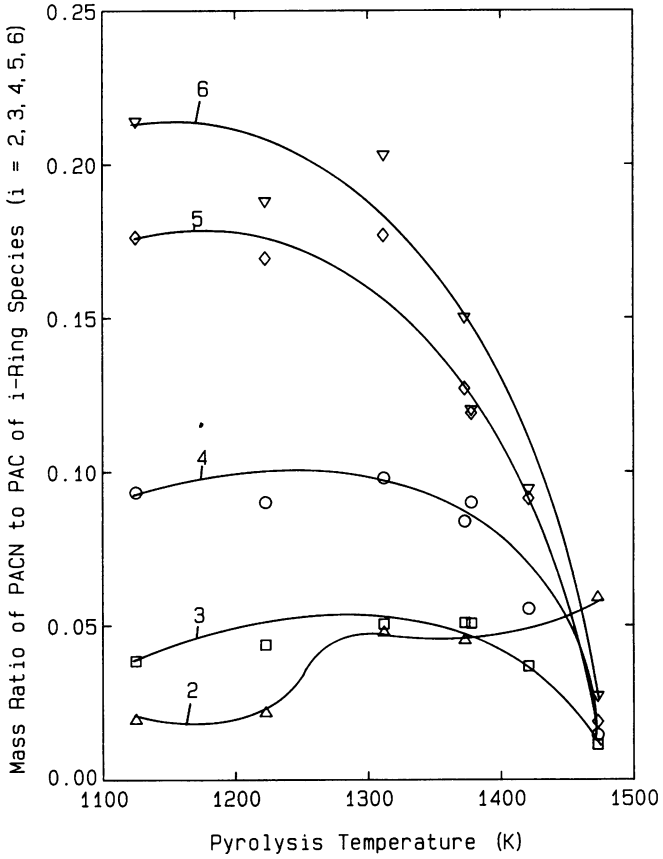

FIGURE 5. The effect of pyrolysis temperature on the ratio of nitrogen-containing PAC to nonnitrogen-containing PAC. Data from Wornat et al. (*28*).

fractions with rising temperature indicates that as pyrolysis temperature increases, the PAH change composition, becoming more mutagenic. Previous chemical analyses (*27*) on the unsubstituted PAC found in our experiments show that, indeed, several changes occur: lower ring number species disappear and larger, more highly condensed ring structures form. Some of the structures that exhibit the highest thermal stability are species known to be highly mutagenic: fluoranthene and cyclopenta[cd]pyrene and perhaps benzo[a]pyrene.

The −PMS activity displayed by the unsubstituted fractions is almost certainly due to PACN. The work of Ho et al. (*39,40*) has exposed the extremely high mutagenicity of several PACN, some of which demonstrate activities significantly higher than that of benzo[a]pyrene. A previous publication (*28*) details the changes in the composition of the PACN from our coal pyrolysis experiments. Of particular interest to our present concerns, however, is how the PACN change with respect to those without nitrogen. Figure 5 portrays the ratio of the PACN to nonnitrogen-containing PAC for each ring number, as functions of pyrolysis temperature. As the figure portrays, the ratio falls with temperature, just as the ratio of −PMS to +PMS activity in our unsubstituted PAC fraction falls as temperature increases from 1223°K. We thus see a consistency between the effects of pyrolysis temperature on the relative proportion of −PMS to +PMS activity and on the relative proportion of nitrogen-containing to nonnitrogen-containing PAC.

Summary and Conclusions

The polycyclic aromatic compounds produced in the pyrolysis of a highly volatile bituminous coal at temperatures of 1125 to 1425°K prove to be mutagenic to *Salmonella typhimurium*, both in the presence and in the absence of PMS. −PMS mutagenicities of unfractionated PAC samples are relatively low for the entire range of pyrolysis temperatures. Though comparable to −PMS mutagenicities at lower temperatures, +PMS mutagenicities become appreciably higher at higher temperatures. When PAC yields are factored into the previously mentioned specific mutagenicities to obtain overall mutagenicities (i.e., per mass of coal consumed), further differences between the −PMS and +PMS activities emerge: Overall −PMS mutagenicity decreases as temperature increases because PAC yield falls, and −PMS specific mutagenicity changes little as temperature is raised. Overall +PMS mutagenicity, on the other hand, peaks at 1378°K, where +PMS specific mutagenicity is highest and just before the abrupt temperature-induced decrease in PAC yield. PAC yield is thus the prevailing factor for overall—PMS mutagenicity.

Using a new HPLC technique that does not alter the mutagenicity of PAC mixtures, we split each coal-

derived PAC sample into two fractions: LC1, containing PAC with alkyl and O-containing substituents and LC2, consisting of unsubstituted PAH and PAC with ring nitrogen. Substituted (LC1) fractions show no significant mutagenicity in the presence of PMS, indicating that as a whole the alkylated PAC in our coal pyrolysis products are not mutagenic. In the absence of PMS, only at the higher temperatures do the substituted fractions exhibit a small but significant mutagenicity, which can be attributed to PAC with carbonyl or etheric functionalities. The extremely low yields of the substituted PAC under the conditions where they show some activity, however, ensure that they contribute little to overall activity.

Unsubstituted (LC2) fractions behave quite differently from their substituted counterparts. As pyrolysis temperature increases, unsubstituted fractions demonstrate a rise in +PMS mutagenicity, attributable to the unsubstituted PAH (and perhaps somewhat to the PACN) in these fractions. −PMS mutagenicity, on the other hand, peaks at a low temperature of 1223°K and falls with increasing temperature. The decline in the −PMS activity and the rise in the +PMS activity of the unsubstituted fractions parallels the previously observed depletion of the PACN (relative to the more thermally stable (PAH) in these samples as temperatuare rises. In contrast to the substituted PAC, the unsubstituted PAC are present in appreciable quantities at all pyrolysis temperatures investigated, so their overall mutagenicity is never negligible. The unsubstituted PAC fractions, comprised of mutagenic PAH and PACN, appear to be responsible for virtually all of the mutagenicity in these coal-derived PAC samples.

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